Supplementary Information

The KDM4/JMJD2 histone demethylases are required for hematopoietic stem cell maintenance

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Supplementary Figure 1: (A) Schematic drawing of the three KDM4 loci in KDM4ABC knockout mice. (B) Recombination efficiency *in vivo* one month after tamoxifen injection measured by qPCR using genomic DNA from cells sorted from peripheral blood. (C and D) Equal levels of H3K9me3 on the TSS of *Nom1* and *Taf1b* in KDM4A and KDM4C single knockout as well as WT LSK cells. LSK cells from mice with the indicated genotypes were FACS sorted, fixed and used in ChIP-qPCR experiments with antibodies against H3K9me3 and H3. The % enrichment relative to H3 are depicted. (E and F) ABI sequencing files of PCR products spanning the cut sites of the gRNAs used in this study. gRNA and PAM sequence are indicated in the sequence. A clear induction of indels are evident downstream the PAM sequence for both Nom1-sg1, Nom1-sg2 (E) and Taf1b-sg1 (F) Taf1b-sg2 did not induce sufficient amount of indels (data not shown) and data using this construct was removed from Figure 2G. (G) LSK cells were sorted from mice expressing Cas9-P2A-EGFP constitutively from the Rosa26 locus. These cells were transduced with RFP-tagged lentiviral sgRNA constructs against *Nom1* and *Taf1b* as well as controls. After transduction the cells were grown for 48hours and subsequently mixed with total BM from WT mice and used in transplantation of lethally irradiated mice. The transduced cells were transplanted into 4 mice per construct and after 4 weeks the GFP/RFP percentage were measured in the LSK fraction from the mice.

Supplementary Figure 2: Combined deletion of *Kdm4b* and *Kdm4c* does not affect hematopoiesis (A) Histogram showing total cell count of nucleated cells in hematopoietic tissues. Bone marrow count represents cells isolated from one femur and tibia. (B) Histogram indicating the fraction hematopoietic stem and progenitor (HSPC) cells of live cells in the bone marrow. LK, $Lin^{-}c\text{-}kit^{+}$; GMP, Granulocyte-monocyte progenitor; MEP, Megakaryocyte-erythroid progenitor; LSK, $Lin\text{-}Sca1^{+}cKit^{+}$. (C) Histogram indicating the fraction of granulocytes ($CD11b^{+}Gr1^{+}$) of total

cells in bone marrow and spleen. (D) Histogram indicating the fraction of erythroid progenitors ($CD71^+Ter119^+$) of total cells in bone marrow and spleen. (E) Histogram indicating fraction of $CD4^+$ single positive (SP) or $CD8^+$SP of total cells in the thymus. (F) Histogram indicating fraction of total B-cells ($B220^+$) or immature B-cell populations in the bone marrow. Pro/Pre^-B, $B220^+IgM^-$; Mature-B, $B220^+IgM^+$. In (A)-(F), data is represented as mean +/-SD (n=4). No significant ($p < 0.05$, two-tailed Student's t-test) differences were observed.

Supplementary methods

MLL-AF9 cells were transduced with SFFV-Cas9-sgRNA vectors containing sgRNA sequences against *Taf1b* and *Nom1*. GFP-positive cells were sorted 2 days after transduction using a BD-FACS Aria III cell sorter (BD Biosciences). Genomic DNA was isolated from the sorted cells 72 h after transduction using the DNAeasy blood and tissue kit (Qiagen). PCR on the genomic DNA was performed using either Taq polymerase (Qiagen) or KAPA Taq polymerase (KAPA Biosystems). Sanger sequencing of the PCR products was performed by Eurofins Genomics using the forward primer of the respective PCR reaction. TIDE analysis was performed using the TIDE software (https://tide.deskgen.com/).

sgRNA sequences (20 bp):

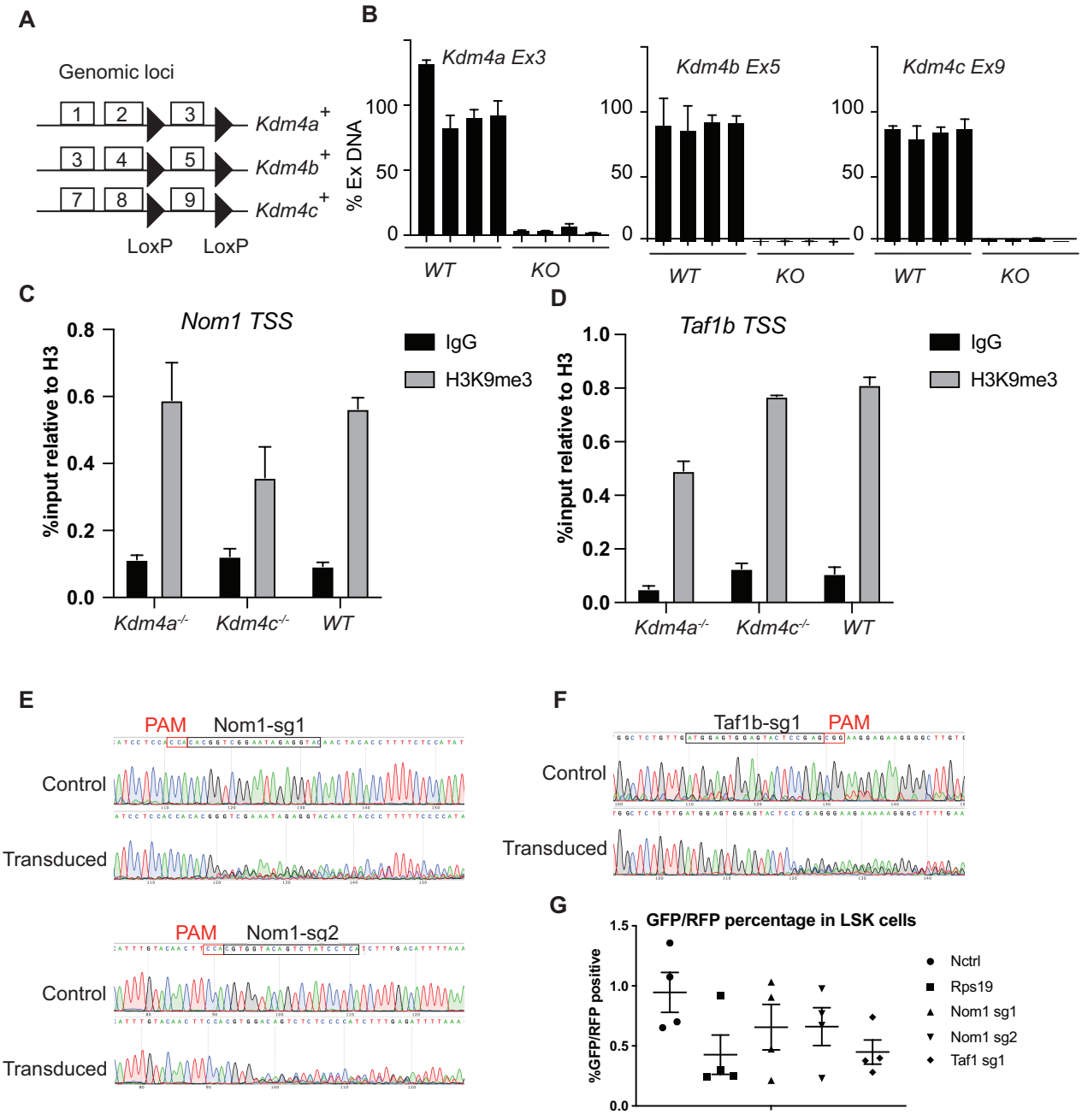
Taf1 sg1: ATGGAGTGGAGTACTCCGAG

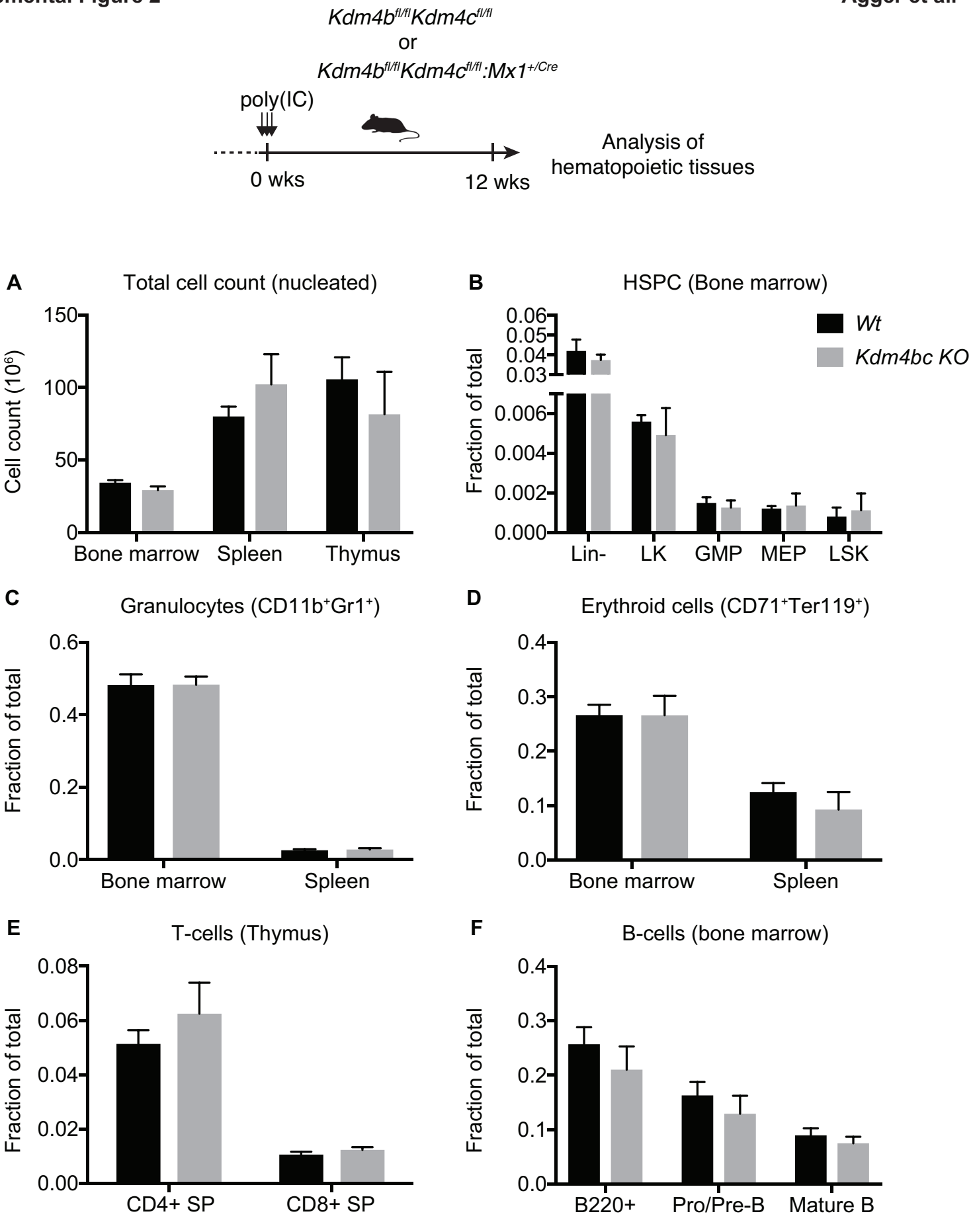
Nom1 sg1: GTACCTCTATTCCGACCGTG

Nom1 sg2: TGAGGATAGACTGTACCACG

	Primer sequences for TIDE analysis (5'-3')		product size
Nom1_sg1_TIDE_F	TAGAGGAGCTGTACATGGCC		319 bp
Nom1_sg1_TIDE_R	GTAGCCAGGGTAGTCTTGCA		
Nom1_sg2_TIDE_F	GTTGGTGCTTGCTTCCTTGA		282 bp
Nom1_sg2_TIDE_R	GGTCTGGGCTTCTGTGATCA		
Taf1_sg1_TIDE_F	TCCACCCTACTCCCACAGAT		240 bp
Taf1_sg1_TIDE_R	AATTGTTTCCCTCTGCCAGA		

Supplemental Figure 1 **Agger et al.**




Kdm4b^fl/fl Kdm4c^fl/fl
or
Kdm4b^fl/fl Kdm4c^fl/fl :Mx1^+/Cre
poly(IC)
0 wks
12 wks
Analysis of hematopoietic tissues
A
Total cell count (nucleated)
Cell count (10^6)
150
100
50
0
Bone marrow
Spleen
Thymus
B
HSPC (Bone marrow)
Fraction of total
0.06
0.05
0.04
0.03
0.006
0.004
0.002
0.000
Wt
Kdm4bc KO
Lin-
LK
GMP
MEP
LSK
C
Granulocytes (CD11b+Gr1+)
Fraction of total
0.6
0.4
0.2
0.0
Bone marrow
Spleen
D
Erythroid cells (CD71+Ter119+)
Fraction of total
0.4
0.3
0.2
0.1
0.0
Bone marrow
Spleen
E
T-cells (Thymus)
Fraction of total
0.08
0.06
0.04
0.02
0.00
CD4+ SP
CD8+ SP
F
B-cells (bone marrow)
Fraction of total
0.4
0.3
0.2
0.1
0.0
B220+
Pro/Pre-B
Mature B